Vol. 10, Nos. 4-6 April-June, 1934 Nos. 115-117

Bulletin of the Agricultural Chemical Society of Japan.

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Published by the
Agricultural Chemical Society of Japan
c/o Faculty of Agriculture, Tokyo Imperial University.

Single Copy (Postage inclusive) :- ¥ 0.35
Annual Subscription (12 numbers) :- ¥ 3.50

The Agricultural Chemical Society of Japan.

President : Umetaro SUZUKI.

The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2, No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

Untersuchungen über die Enzyme von Bombyx mori L. VI. Mitteilung.

Über die Blutlipase.

Von

Kazuo YAMAFUJI.

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(Eingegangen am 5, Januar 1934)

Der erste Versuch zu einer quantitativen Bestimmung der Blutlipase bei höheren Tieren findet sich in der Arbeit von Rona und Michaelis[1], die die Spaltung von Mono- oder Tributyrin durch das Ferment mit der stalagmometrischen Methode verfolgten.

Was Insekten betrifft, so ist vielleicht zum erstenmal vom Verf. ein solcher Versuch gemacht worden und im nachstehenden möchte er die Ergebnisse seiner Untersuchungen über die Blutlipase von Bombyx mori mitteilen.

1) *Methodik*:- Wenn nicht anders angegeben, wurden 25 ccm gesättigte wässerige Tributyrinlösung, 2.5 ccm M/3 Phosphatpufferlösung von Ph 6.8 und 0.5 ccm Blut vermischt. Je nach 0, 10, 20 und 30 Minuten Erwärmung bei 20° wurde die Abspaltung von Tributyrin mit dem Stalagmometer ermittelt. Die Wirksamkeit der Blutlipase wurde mit den Konstanten, die nach der Gleichung der monomolekularen Reaktion berechnet wurden, verglichen.

2) *Optimale Ph.*

Larve, ♀		Larve, ♂		Puppe, ♀	
Ph	$k.10^2$	Ph	$k.10^2$	Ph	$k.10^2$
5.3	0.48	6.5	0.53	7.0	0.83
6.2	0.67	6.8	0.98	7.4	0.85
7.0	1.20	7.2	1.12	7.7	0.96
8.0	1.18	7.7	1.49	8.0	0.73

3) *Optimaltemperatur.*

Larve, ♀, Ph=6.8		Puppe, ♂, Ph=7.7	
Temp.	$k.10^2$	Temp.	$k.10^2$
5	0.56	20	0.33
20	1.23	30	0.77
35	3.46	40	1.16
50	3.07	50	1.05

4) Kinetik.

Bei Puppe: 50 ccm gesätt. Tributyrinlösung + 1.5 ccm Pufferlösung + 0.5 ccm Blut, Ph = 7.7, Temp. = 20°.

Zeit, Minuten	Larve, ♀, Ph = 6.8		Puppe, ♀, Ph = 7.7	
	a − x	k.10²	a − x	k.10²
0	73	—	73	—
10	47	1.91	60	0.85
20	31	1.86	50	0.82
30	20	1.87	41	0.84
40	—	—	34	0.83
50	—	—	28	0.83
60	—	—	24	0.81

a − x bedeutet die in der Zeit t noch vorhandene Menge der Tributyrins (gesättigte wässerige Lösung = 100).

Puppe, ♀, Ph = 6.8		Puppe, ♂, Ph = 7.7	
Fermentmenge, ccm	k.10²	Fermentmenge, ccm	k.10²
0.25	0.47	0.20	0.48
0.50	0.88	0.40	0.91

5) Einfluss von Chinin oder Atoxyl.

2 ccm Pufferlösung + 0.5 ccm Blut + 1 ccm Giftlösung. Nach 30 Min. Zugabe von 50 ccm Tributyrinlösung.

♀, Ph = 7.7		♂, Ph = 6.8		♀, Ph, = 7.7		♀, Ph = 6.8	
Chinin, mg	k.10²	Chinin, mg	k.10²	Atoxyl, mg	k.10²	Atoxyl, mg	k.10²
0	0.79	0	0.72	0	0.43	0	0.95
5.0	0.58	2.5	0.64	5.0	0.38	2.5	0.64
10.0	0.41	5.0	0.53	10.0	0.36	5.0	0.55

6) Unterschied zwischen gut gewachsenen und schlecht gewachsenen Raupen.

	Gut gewachs. Raupen		Schlecht gew. Raupen	
	♀	♂	♀	♂
k.10²	1.11	0.99	1.14	1.06

7) Änderungen durch Hunger bei der Larve.

	Hungerstund.	1	23	47
k.10^2	♀	0.72	0.84	1.27
	♂	1.09	1.06	1.40

8) Unterschied zwischen gesunden und kranken Raupen.

	Gesunde Raupen		Krank. Raup. (Nankabyo)	
	♀	♂	♀	♂
k.10^2	1.52	1.06	1.58	1.36

9) Veränderungen im Laufe der drei Entwicklungsperioden von Bombyx mori.

		k.10^2	
	Tage	♀	♂
Larve	V. Lebensalter 3	0.86	0.93
	V. Lebensalter 6	0.72	1.09
	V. Lebensalter 8	1.14	1.15
Einspinnen des Kokons	2	1.89	2.19
	5	2.00	1.76
Puppe	1	1.70	1.71
	3	1.41	1.55
	7	0.95	1.18
	12	0.82	0.60
Schmetterling	1	0.79	—

10) Diskussion der Versuchsresultate.

Wie oben erwähnt, habe ich in meiner vorliegenden Arbeit über einige Eigenschaften der Blutlipase von Bombyx mori geforscht und aufgeklärt, dass diese Lipase in ihrer enzymatischen Natur derjenigen des Blutes höherer Tiere sehr nahe steht.

Aber über die Herkunft der Blutlipase bei der Seidenraupe können wir wie bei höheren Tieren nichts sagen, denn wir haben keine eingehenden Angaben über die Organlipasen bei diesem Insekt.

Darauffolgend kommt die Frage der biologischen Bedeutung der Blutlipase.

Bei den höheren Tieren scheint die Blutlipase nichts mit der normalen

Ernährung zu tun zu haben. Aber in der fünften Mitteilung[2] habe ich bereits über die engen Beziehungen zwischen den Blutdisaccharasen bei der Seidenraupe und den chemischen Umsetzungen der Disacharosen im Körper derselben gesprochen. Diese Beziehung der Blutfermente von Bombyx mori zum Stoffwechsel behalten auch für die Blutlipase ihre Geltung.

Das Maulbeerblatt enthält kein echtes Fett, dagegen die Seidenraupe immer beträchtliche Mengen Körperfett aufzuweisen hat. Im Digestionssafte der Seidenraupe kommt keine oder nur wenig Lipase vor, im Blut derselben indessen eine verhältnismässig wirksame Lipase. Bei Hunger ist die Blutlipase vermehrt. Die Lipase der kranken Raupe ist wirksamer als die der gesunden. Eine erhebliche Anlagerung des Körperfetts geschieht im fünften Lebensalter der Larve. Die Puppe und der Schmetterling leben hauptsächlich auf eigene Kosten des Körperfettes. Im Laufe von drei Entwicklungsperioden von Bombyx mori wirkt die Blutlipase in den Tagen des Kokonspinnens und des Verpuppens am aktivesten.

Aus den oben angeführten Data geht hervor, dass die Blutlipase bei Bombyx mori in der Assimilation oder beim Abbau des Fetts eine wesentliche Rolle spielt.

Zusammenfassung.

(1) Die Spaltung des Tributyrins durch die Blutlipase von Bombyx mori kann in guter Annäherung mit Hilfe der Gleichung der monomolekularen Reaktion ausgedrückt werden.

(2) Die Blutlipase zeigt die optimale Aktivität bei Ph 7.7 ; ihr Temperaturoptimum liegt bei 40°.

(3) Die Lipase ist gegen Chinin oder Atoxyl wenig empfindlich.

(4) Die Wirkung der Lipase ist bei kranken Raupen (Nankabyosan) viel höher als bei gesunden und auch bei schlecht gewachsenen Raupen etwas stärker als bei gut gewachsenen. Durch Hunger wird sie gesteigert.

(5) Die Blutlipase wird im fünften Lebensalter der Larve mit dem Wachstum stärker, die ihr Maximum in den Tagen des Kokonspinnens und des Verpuppens erreicht und dann ist sie in den Perioden der Puppenruhe und des Schmetterlingslebens allmählich vermindert. Es besteht kein wesentlicher Geschlechtsunterschied in der Lipasewirksamkeit.

(6) Bei Bombyx mori scheint die Blutlipase am Fettstoffwechsel Anteil zu nehmen.

Zum Schluss danke ich herzlich Herrn Prof. Y. Okuda für seine Anregung zu diesen drei Arbeiten.

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Nutritive Value of Canavanin (amino acid)

By

M. Ogawa.

(Received February 1, 1934)

A new amino acid called Canavanin was discovered and named by Dr. Kitagawa of Fukuoka Imperial University in 1929. But none has been investigated on the nutritive value of that amino acid yet. Therefore the author tried to determined the nutritive value of that amino acid.

In this experiment, the author employed a number of young male albino rats weighing about 42 grams. Divided the animals into 4 groups, and fed them on a diet deficient in Canavanin. To the group I, gave Canavanin 0.1 gram per Kilo-gram of the body weight per day. To the group II, gave Canavanin 0.05 gram per Kg. of the body weight per day, and to the group III, gave Canavanin 0.01 gram per Kg. of the body weight per day. But to the fourth group, did not give any Canavanin, on account of this group was a control.

The author fed the animals as mentioned in the above way for 40 days, and obtained the following results.

1. The mortality of the animals fed on Canavanin was rather lower than the animals which was not administered Canavanin. Therefore Canavanin is not poisonous or harmful substance.

2. The animals administered Conavanin had a flow of spirits, while the animals which did not received Canavanin were rather depression of spirits.

3. The animals administered Canavanin were grew better and increased the body weight more rapidly than the animals which did not consume Canavanin.

4. Regard to the amount of the consumption of diet, the animals administered Canavanin was smaller amount than the animals which were not administered Canavanin.

Researches on the Electrolytic Reduction Potentials of Organic Compounds. Part 18.

On the electrolytic reduction of camphor, again.

By

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(Agricultural Chemical Institute of the Department of Agriculture, Kyoto Imperial University)

Abstract from original paper.

(Recived March 1, 1934)

The polarographic study of camphor was previously performed by Prof. M. Shikata and M. Watanabe. The authors found that the polarograms of camphor had generally two reduction waves.

Their reduction potentials were independent on hydrogen-ion concentration of electrolyzing solutions and their saturation currents were much smaller than other various compound in same condition.

The present author studied on the electrolytic reduction of camphor again to explain the mechanism of the appearance of abnormal reduction waves.

He found that three waves appeared on the polarogram obtained from 0.1 n HCl containg 10^{-2} m camphor.

He discussed the phenomena after cansideration of electrocapillar curve and concluded as follows.

(1) The first wave is not due to the true reduction but due to the replacement of Cl' to camphor molecule on the electrode surface.

(2) The second wave is the true reduction wave, which potential shifted by the change of pH as expected by Nernst's formula.

(3) The third wave is due to the destroy of molecular camphor on the electrode surface.

Hochsiedende unverseifbare Substanz des Fuselöls (II. Mitteilung)

Von

T. Taira und T. Masuzima.

(Eingegangen am 1, März 1934)

Der eine der Verf. (T. Taira)[1] hat in der vorigen Mitteilung darauf hingewiesen, dass er aus dem hochsiedenden Anteil des Rohrzuckermelassefuselöls Methylheptylcarbinol isoliert und im Ipomoeafuselöl ausser den beiden vermuteten sekundären Alkoholen Phenyläthylalkohol nachgewiesen hat. Ferner hat er Phenyläthylalkohol im Reisbranntweinfuselöl und Sake isoliert und festgestellt. Auch hat er[2] vor mehreren Jahren mitgeteilt, dass er aus dem hochsiedenden unverseifbaren Anteil des Reisbranntweinfuselöls sesquiterpen- und diterpenartige Kohlenwasserstoffe isolieren konnte. Die vorliegende Untersuchung befasst sich mit den hochsiedenden, unverseifbaren Anteil der Ipomoea- und Rohrzuckermelassefuselöle sowie andrer Fuselöle. (Eine eingehende diesbezügliche Mitteilung ist gegenwärtig unter Druck und zwar im Journ. Agr. Chem. Soc. of Japan).

I. n-Heptylalkohol.

Faget[3] hat aus der Tatsache, dass n-Heptylsäure unter den Oxydationsprodukten des Weintreberfuselöls vorkommt, geschlossen, dass darin n-Heptylalkohol enthalten ist. Der so erhaltene Heptylalkohol vom Sdp. 155~160° ist nach ihm von dem durch Reduktion des Oenanthol gewonnenen n-Heptylalkohol vom Sdp. 175° verschieden. Später wurde Heptylalkohol von Windisch[4] im Kornfuselöl, von Claudon und Morin, von Ordonneau[5], von Ost[7] im alten Kognak vesmutet. Auch in Japan vermuteten einige Autoren,[8][9][10] dass n-Heptylalkohol in einigen Fuselölen und im Reisbranntwein enthalten ist. T. Taira hat zwar in der vorigen Mitteilug darauf[1] hingewiesen, dass er beim Nachweis der sekundären Alkohol im Rohrzuckermelassefuselöl den primären Alkohol nicht finden konnte, doch haben diesmal Verf. zur Entscheidung der Frage, ob n-Heptylalkohol enthalten ist oder nicht, die beiden folgenden Versuche angestellt:

(A) Nach Destillation des unterhalb 132° siedenden Anteils aus 54 Liter Rohrzuckermelassefuselöl unter gewöhnlichem Druck wurde der unverseifbare Teil getrennt, dann wurde Phtalsäureanhydrid auf die unter 80 mm zwischen 105~130° siedende, 64 ccm betragende Fraktion in Benzollösung einwirken lassen. Der Phtalsäureester des primären Alkohols wurde mit Alkali abgeschieden, mit überschüssigem Alkali verseift, der unverseifbare Teil mittels

Äther getrennt und der fraktionierten Destillation unterworfen, wobei nur eine geringe Menge der den Methylamylcarbinol und Phenyläthylalkohol entsprechenden Fraktion, aber kein n-Heptylalkohol erhalten wurde.

(B) Nach gleichem Verfahren wurde der hochsiedende, unverseifbare Anteil aus 21 Liter Rohrzuckermelassefuselöl vom Amylalkohol getrennt. 20 ccm der uner gewöhnlichem Druck zwischen 160~180° siedenden Fraktion wurden mit Phtalsäureanhydrid behandelt und die primären Alkohol isoliert, aber ein mit dem Siedepunkt 175° des n-Heptylalkohols übereinstimmender Anteil nicht erhalten. Nur eine sehr geringe Menge Substanz, die nach sekundärem Alkohol roch, wurde erhalten.

Der Siedepunkt des von Faget[13] zuerst isolierten Heptylalkohols stimmte mit dem des Methylamylcarbinols ueberein. Verf. vermuten, dass die von ihm erhaltene n-Heptylsäure vom Methylheptylcarbinol stammt.

II. Phenyläthylalkohol des Rohrzuckermelassefuselöls.

In der vorigen Untersuchung konnte Phenyläthylalkohol im unverseifbaren Anteil des Ipomoeä- sowie Reisbranntweinfuselöls nachgewiesen werden, aber die dem Phenyläthylalkohol entsprechende Fraktion des Rohrzuckermelassefuselöls bildete zwar Diphenylharnstoff, aber Urethan konnte in befriedigender Weise nicht dargestellt werden. Die beiden Fraktionen vom Sdp. 120~130° unter 40 mm und 120~135° unter 20 mm aus 54 Liter Fuselöl wurden vereinigt und nach der oben erwähnten Methode wurden mittels Phtalsäureanhydrid die primären Alkohole aus 160 ccm der Mischung isoliert, wodurch eine farblose und klare, etwas nach sekundären Alkoholen riechende, dabei einen starken Rosengeruch aufweisende Fraktion von 60 ccm mit dem Sdp. 120~126° unter 40 mm erhalten wurde. $d\,\frac{20°}{4°} = 1{,}0072$. Das in üblicher Weise hergestellte und aus Alkohol umkristallisierte Phenylurethan hatte den Schmp. 80° und die Mischprobe zeigte Keine Depression. Die Stickstoffbestimmung nach Dumas ergab 5,9% N; dieser Gehalt mit der berechneten Menge ($C_{15}H_{15}O_2N$) von 5,81% fast ueberein.

III. Sesquiterpenalkohole.

(A) Sesquiterpenalkohol aus Ipomoeafuselöl.

Beim Fraktionieren des hochsiedenden, unverseifbaren Anteils des Ipomoeafuselöls wurde eine schön blaue, zwischen 140~150° unter 5 mm siedenden Fraktion in einer etwa 0,3% des Fuselöls entsprechenden Ausbeute erhalten. Dieselbe ging bei erneuter Fraktionierung unter 5 mm zwischen 143~145° über. Beim Erhitzen mit metallischem Natrium verharzte die ganze Flüssigkeit und auch bei Wiederholung der Fraktionierung verharzte ein Teil derselben. Diese wohlriechende Flüssigkeit zeigte die Liebermannsche Reaktion und

entfärbte Brom. Die Analysenzahlen stimmten annähernd mit der Formel $C_{15}H_{24}O$. Durch Erhitzen mit der alkalischen Lösung der Diazobenzolsulfosäure trat eine Rotfärbung auf. Sdp./760 mm = 320°. $d_{4^\circ}^{20^\circ} = 0{,}9367$. $n_D^{20} = 1{,}5052$. $\alpha_D = +1.8$. Mol. Refraktion: Gef. 69.61. Ber. ($C_{15}H_{24}OF_2$) = 67,67. Das Resultat wies auf eine bicyklische Verbindung hin. Durch Hydrierung bei Gegenwart von $PdCl_2$ oder Platinschwarz wurde der Wasserstoff nicht aufgenommen. Durch Bromierung nach der Methode von Allen addierte 1 Mol der verbindung 3 Atom. Brom. Weder durch Kochen der Verbindung in Benzollösung mit Phtalsäureanhydrid noch durch direktes Erhitzen mit dem letzteren auf etwa 140° trat eine Reaktion ein. Auch Phenylisocyanat reagierte nicht. Die Reaktion von Denigés auf tertiären Alkohol war positiv. Die Substanz gab keine Kristalle mit Chlorwasserstoff, Bromwasserstoff und Nitrosylchlorid. Die Dehydrierung mit Schwefel oder Selen führte nur zu Harzen und weder Eudalin noch Cadalin war nachweisbar. Durch Dehydrierung mit Ameisensäure oder Kaliumbisulfat verharzte der grösste Teil und nur geringe Mengen Kohlenwasserstoffe, deren Kp. bei etwa 260° und 300° lagen, wurden erhalten. Da dieser Sesquiterpenalkohol von der aus Rohrzuckermelassefuselöl erhaltenen Verbindung deutlich differierte, dessen Untersuchung sich Verf. Vorbehalten.

(B) Sesquiterpenalkohol aus Rohrzuckermelassefuselöl.

Auch aus Rohrzuckermelassefuselöl wurde eine mit Sesquiterpenalkohol des Ipomoeafuselöls ähnliche Fraktion in einer 1% des Fuselöls betragenden Ausbeute gewonnen. Die Farbe war nicht so tief wie beim Ipomoeafuselöl, sondern sehr schwach gelblich und unbeständig. Sdp./760 mm = 320°. $d_{4^\circ}^{20^\circ} = 0{,}9228$. $n_D^{26} = 1{,}5018$. $\alpha_D = -1{,}8$. Mol. Ref: gef. 70,50; ber. ($C_{15}H_{24}OF_2$) 67,67. Dieselbe zeigte die Reaktion des tertiären Alkohols. Bei der katalytischen Reduktion wurde der Wasserstoff nicht addiert. Bei der Bromierung nach Allen wurden 4 Atom. Brom addiert. Die Dehydrierung gab auch die gleichen Resultate wie beim Sesquiterpenalkohol des Ipomoeafuselöls d. h. eine geringe Menge zweier Kohlenwasserstoffe. Da bei der Liebermannschen Reaktion eine deutliche Verschiedenheit bemerkbar war.

IV. Diterpen.

Durch Erhitzen und Destillation der Fraktion (Sdp. 150~180° unter 5 mm) aus dem unverseifbaren Teil des Ipomoea- und Rohrzuckermelassefuselöls mit metallischem Natrium bekamen Verf. einen farblosen, klaren und fast geruchlosen Kohlenwasserstoff. Die Liebermannsche Reaktion gab keine deutliche Färbung. Der Kohlenwasserstoff entfarbte nicht Brom. Gegen Oxydationsmittel verhielt er sich beständig. Das Molekulargewicht stimmte mit Diterpen überein. Die folgende Tabelle enthält die Konstanten des Diterpen

aus den beiden oben erwähnten Fuselölen und Reisbranntweinfuselöl[2]:

	Ipomoeafuselöl	Rohrzuckermelasse-fuselöl	Reisbranntwein-fuselöl
Kp./760 mm	355°	360°	355°
$d_{4^\circ}^{20^\circ}$	0,9310	0,9141	0.9150
$n_D^{20^\circ}$	1,5170	1,5100	1,5120
α	0	0	0
Mol. Ref. gef.	88,39	88,98	89,21
" " ber.	$C_{20}H_{32}F_3$ 88,76		

V. Cetylalkohol.

Beim Abkühlen der Diterpenfraktion aus Ipomoea- und Rohrzucker melassefuselöl bildeten sich Kristallblättchen. Als Verf. die Lösung dieser Kristalle in gleichem Volumen Äthylacetat mit einer Kältemischung genügend abkühlten, dann rasch abfiltrierten und die Substanz aus Äthylacetat umkristallisierten, erhielten sie die Verbindung vom Schmp. 49°. Dieselbe hat nach der Analyse die Zusammensetzung $C_{16}H_{34}O$. Die Mischprobe mit reinem Cetylalkohol veranlasste keine Schmelzpunktserniedrigung. Durch Einwirkung von Phenylisocyanat in Benzollösung wurden seidenglänzende Kristalle vom Schmp. 76° erhalten. Die Mischprobe mit Phenylurethan zeigte keine Depression. Nach Dumas analysiert wurde der Stickstoffgehalt 3,93% gefunden. Derselbe stimmt mit dem auf $C_{23}H_{39}NO_2$ berechneten Stickstoffgehalt 3,90% überein. In der Pflanzenwelt wurde Cetylalkohol bisher nur in Dorema ammoniak, Don gefunden, daher ist es vom grossen Interesse, dass derselbe auch im Fuselöl vorkommt.

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Studies on the Proteins Contained in Mulberry Leaves. Part IV.

A Comparative Study of the Digestibility of the Proteins Contained in Mulberry Leaves

By

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(At the Katakura Research Institute of Mulberry Culture, near Hachioji City, Tokyo Prefecture, Japan)

(Received April 5, 1934)

Résumé

(1) I stated in Parts II and III[1] that the proteins (considered collectively) contained in mulberry leaves undergo great change in quality with the growth of the leaves, or, in other words, each protein undergoes more or less change in quantity; and also that the absolute quantity of the proteins contained in the silkworm-body and in cocoon silk is always found to increase in proportion to the degrees of youthfulness of the mulberry leaves used in feeding the silkworms.

Now, here in Part IV, I have made the following studies concerning the digestibility of the proteins contained in mulberry leaves: –

(2) As the results of experimentation (described below) in artificial digestion made with the gastric juice of silkworms, I have found that the digestibility of all the proteins (considered collectively) contained in mulberry leaves varies according as the proteins change in quality with the growth of the leaves, or, to put it in plain words, the proteins contained in younger leaves are easier of digestion than the proteins contained in older ones.

The results of the experiments are as follows: –

(a) Experiments in artificial digestion with the gastric juice of silkworms have demonstrated that the protein which dissolves in the boiling 60% alcohol containing 0.3% of sodium hydroxide and which increases, with the growth of the leaves, its proportion to the total quantity of all the proteins in mulberry leaves, is less digestible than any other protein contained in the leaves and seeds of mulberry plants.

(b) When gastric juice is applied to well-crushed younger and older leaves (to which a buffer solution containing pH 9.8 has previously been applied) respectively, the digestibility of the proteins contained in the

younger leaves is found to be superior to that of the proteins contained in the older ones; while, when only the buffer solution is applied to the younger leaves, the pH value of the liquid thus obtained is found to be slightly inferior to that of a liquid similarly made from the older leaves; also, within the range of pH 9.30 to pH 9.86, which covers the scope of these experiments, it is found that the proteins contained in mulberry leaves fall in digestibility with the fall of pH values. Moreover, digestion experiments made upon each of the sample solutions that have previously been all brought to the same pH value (i. e. 9.8), also show that the digestibility of the proteins contained in the younger leaves is superior to that of the proteins contained in the older ones.

(c) I wish to add that these experiments have been made exclusively either on mulberry leaves with cell-walls well crushed, or on sap pressed out of well-crushed fresh leaves.

(3) Digestion experimets made in actual silkworm culture show the same varying degrees of digestibility observable in the proteins contained in mulberry leaves, just as in the case of the artificial digestion experiments described above. Also, the results of the silkworm culture practised in this way are found to be identical with those stated in Part III.

(4) To sum up, as the proteins contained in mulberry leaves undergo change in quality with the growth of these leaves, they also present different degrees of digestibility. In other words, the digestibility rises in proportion to the degrees of youthfulness of the leaves used in feeding the silkworms.

(5) As a result of experimentation on silkworms that have been fed on mulberry leaves in different stages of growth, it has been discovered that, when a group of silkworms have been fed on younger leaves containing a given quantity of proteins and another group on older leaves containing the same quantity of proteins, a larger proportion of the proteins contained in the younger leaves has been ingested than of the proteins contained in the older leaves. The superiority of the proteins contained in younger leaves to those in older leaves is thus proved again in this experiment, just as it was in the digestion experiments described above.

Also, the digestibility and the proportion that has been ingested of the dry matter in the leaves are found to be similar to those of the proteins contained in the leaves.

Literature.

(1) Y. Kishi: Bull. of Agricul. Chem. Soc. of Japan, Vol. IX, Nos. 1—3, 1933.

Studies on the Proteins Contained in Mulberry Leaves. Part V.

On the Quantitative Changes of All the Proteins (Considered Collectively) Contained in a Given Quantity of Fresh Mulberry Leaves, as Considered with Relation to the Growth of these Leaves.

By

Yukitaro KISHII and Yonekichi YOKOTA

(At the Katakura Research Institute of Mulberry Culture, near Hachioji City, Tokyo Prefecture, Japan)

(Received April 5, 1934)

Mésumé

(1) Here we have studied how all the proteins (considered collectively) contained in a given quantity of fresh mulberry leaves, undergo change in quantity with the growth of these leaves.

(2) As mulberry leaves come to, or near, the end of their growth due to the stems reaching an advanced stage of growth, it is found that the total quantity of all the proteins contained in a given quantity of fresh leaves becomes generally amaller than the total quantity of those in unmatured younger leaves, which is in the same way as that proteins contained in a given quantity of the dry matter in the leaves undergo change in quantity with the growth of these leaves.

However, this change in the total quantity of all the proteins contained in a given quantity of fresh leaves has been observed to be variable according to the different stages of the leaves and stems, the varieties of the plants, and other conditions of growth.

(3) One of us, who, last year, made studies concerning the proteins contained in mulberry leaves and the culture of silkworms on mulberry leaves in various stages of growth, states in Part III of these Studies that the absolute quantity of the proteins contained in the silkworm - body and in cocoon silk is always found to increase in proportion to the degrees of youthfulness of the mulberry leaves used in feeding the silkworms. This year, again, he has made an inquiry into the cause of the results mentioned in Part III, and reports the results of his experiments in Part IV, to the effect that the digestibility of the proteins contained in younger leaves is superior to that of the proteins contained in older ons, and that this digestibility rises or falls according as the proteins contained in these leaves

change in quality with the growth of the leaves.

Now, on the other hand, we wish to point out that another cause that has brought about the above-mentiond results must be that all the proteins (considered collectively) contained in fresh leaves change in quantity with the growth of the leaves, namely, that the absolute quantity of the proteins contained in the younger leaves used in feeding the silkworms is larger than that of the proteins contained in the older ones.

The writers of these pages wish to express their sincere gratitude to Professor Dr. Y. Okuda for his kind advice throughout these works (Parts IV & V).

ERRATA

to

"Studies on the Proteins Contained in Mulberry Leaves" (Part I~III), by Y. Kishi, in Vol. IX, Nos. 1~3, of this Journal.

Page	Line	Error	Correct
38	margin	*Osborne,	(1)Osborne,
39	2	Was prepared for the purpose of estimating	was obtained when I estimated
40	7 (in table)	in capable	incapable
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42	2	containe	cpntained
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43	11~12 (in table)	experiments	experiment
〃	22	hot alcohol	hot alkaline alcohol
44	19	of the proteins	the absolute quantity of the proteins
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〃	30	weignt	weight
〃	32	silkwoam-body	silkworm - body
〃	34	immaturity	youthfulness

Isolation of Oryzanin (Antineuritic Vitamin) Third Report.

By

Sator Ohdake.

(Agricultural Chemical Laboratory, Faculty of Agriculture, Tokyo Imperial University, Komaba, Tokyo.)

(Received March 29th, 1934)

The author has recently observed that the vitamin-B_1 content of yeast can be increased considerably by suspending fresh living yeast in a solution containing vitamin-B_1 and by passing air continuously for 5～6 hrs. The material used in this experiment was prepared in this way from pressed baker's yeast and the B_1-activity was about ten times greater than common brewer's yeast.

(I) 142 kg of this material (42.790 g of dry-yeast) were extracted with water acidulated with sulphuric acid and treated with acid-clay. The activated solid thus obtained, was now extracted with baryta water. After the removal of baryta with sulphuric acid, the filtrate was concentrated to a small volume and treated with 85% alcohol to remove protein and other impurities. The alcoholic solution was then evaporated to a syrupy consistency which contained 44.2 g. of solid matter and possessed highly activity even in the dose of 0.5 mg for pigeon. This concentrate was now dissolved in 1.500 cc of water and precipitated fractionally with silver nitrate and baryta. The precipitates obtained at the Ph. 4.5～6.8 were decomposed with dilute hydrochloric acid and purified by the phosphotungstic method. The filtrate from barium phosphotungstate was freed from baryta with sulphuric acid, evaporated, then dissolved in absolute alcohol, and concentrated to a small volume after acidifying with hydrochloric acid. The resultant, mostly consisting of the crystalline hydrochloride, was dissolved in a small volume of alcohol and added cautiously with acetone until white turbidity is produced. The crystals, separated out gradually, were collected after keeping in a refrigerator and purified by repeated recrystallisation from alcohol and acetone. Yield :– 0.7 g.

(II) The same material was extracted with 60% alcohol and treated in the same manner as above. The yield was 0.15 g from 20 kg of pressed yeast (=approximately 6 kg of dry-yeast).

(III) In the third experiment, the alcoholic extract was directly fractionated with silver nitrate and baryta and proceeded in the same manner as above. The yield of purified crystals was 0.25 g from 20 kg of pressed

yeast.

Properties of the hydrochloride:- The purified crystals of the hydrochloride form colorless long plates, melting at 249~250°C (uncorr.) with decomposition. Mixed with the preparation from rice-polishings, no depression was observed. Its general reactions, behaviors toward various solvents and precipitants, as well as properties were proved to be exactly identical with the hydrochloride of "oryzanin" isolated by the author from rice-polishings as shown in the following table;

Reagents	Oryzanin hydrochloride	
	from yeast.	from rice-polishings.
Solvents:-		
Water, Alcohol, Methylalcohol, etc.	sol	sol
Acetone, Benzene, Ether, etc.	insol	insol
Precipitants:-		
Phosphotungstic acid	ppt	ppt
Mercuric chloride	ppt	ppt
Silver nitrate & baryta	ppt	ppt
Gold chloride	ppt	ppt
Platinum chloride	ppt	ppt
Picrolonic acid	ppt	ppt
Iodin-potassium-iodide	ppt	ppt
Dragendorff's reagent	ppt	ppt
Mercuric sulphate	nil	nil
Lead acetate	nil	nil
Picric acid	nil	nil
Tannic acid	nil	nil
Fravianic acid	nil	nil
Color reactions:-		
Pauly's diazo-reaction	yellow*	yellow*
Ninhydrin	nil	nil
Biuret-reaction	nil	nil
Sakaguchi's (arginin) reaction	nil	nil
Bial's pentose reaction	nil	nil
Purin reactions	nil	nil
Sulphur reaction (direct)	nil	nil
After boiling with alkali, (or fusing with metallic natrium)		
Lead acetate	black	black
Natrium nitro-prusside	violet	violet
After fusing with kalium nitrate and natrium carbonate		
Barium chloride	white ppt	white ppt

* Differs from the proper reaction.

Analysis of the hydrochloride :–

Found No.	Subst mg	CO_2 mg	H_2O mg	C %	H %	N %	S %	Cl %
[I]	Mpt. 249~250°C (uncorr)							
(1)	4.351	6.516	2.121	40.84	5.42	—	—	—
(2)	4.231	6.371	2.135	41.07	5.60	—	—	—
(3)	Dried 30 mts. at 160–170°C. 4.576	6.938	2.258	41.35	5.48	—	—	—
(4)	Dried 30 mts. at 160–170°C. 4.302	6.492	2.189	41.15	5.65	—	—	—
(5)	4.608	0.618 cc N (18°C 762 mm)		—		15.77	—	—
(6)	4.362	0.584 cc N (17°C 762 mm)		—		15.80	—	—
(7)	4.413	3.043 mg $BaSO_4$		—	—	—	9.46	—
(8)	6.153	4.086 mg $BaSO_4$		—	—	—	9.12	—
(9)	4.774	3.962 mg AgCl		—	—	—	—	20.52
(10)	4.584	3.791 mg AgCl		—	—	—	—	20.45
[II]	Mpt. 249°C (uncorr)							
(11)	4.050	6.107	2.096	41.12	5.75	—	—	—
(12)	4.346	0.594 cc N (17°C 759 mm)		—		15.94	—	—
(13)	4.943	3.322 mg $BaSO_4$		—	—	—	9.23	—
(14)	4.291	3.471 mg AgCl		—	—	—	—	20.01
[III]	Mpt. 249°C (uncorr)							
(15)	4.319	6.404	2.119	40.44	5.45	—	—	—
(16)	4.914	7.346	2.351	40.77	5.32	—	—	—
(17)	4.289	0.569 cc N (16°C 758 mm)		—		15.63	—	—
(18)	4.549	0.629 cc N (19°C 758 mm)		—		16.11	—	—
(19)	4.868	3.373 mg $BaSO_4$		—	—	—	9.52	
(20)	5.945	3.966 mg $BaSO_4$		—	—	—	9.16	—
(21)	4.622	3.815 mg AgCl		—	—	—	—	20.41
(22)	4.333	3.602 mg AgCl		—	—	—	—	20.55
Calc. for $C_{12}H_{16}N_4SO_2$–2HCl				40.79	5.10	15.86	9.07	20.11
Calc. for $C_{12}H_{18}N_4SO_2$–2HCl				40.56	5.63	15.78	9.01	20.00

As there was no loss in weight by drying at 160~170°C for $\frac{1}{2}$ hrs., it is very probable that the crystals have no water of crystallisation.

Picrolonate :– Light yellow needles or prisms, melting sharply at 227°C (uncorr.) with decomposition and no depression was observed when mixed with the preparation from rice-polishings. Dried at 100°C in vacuo and analysed ;

Found No.	Subst. mg	CO_2 mg	H_2O mg	C %	H %	N %	S %
(1)	4.131	7.181	1.491	47.41	4.01	—	—
(2)	4.592	8.005	1.676	47.54	4.05	—	—
(3)	4.339	0.748 cc N (16°C 758 mm)		—	—	20.31	—
(4)	4.376	0.754 cc N (16°C 758 mm)		—	—	20.29	—
(5)	4.245	0.738 cc N (17°C 759 mm)		—	—	20.43	—
(6)	8.751	2.278 mg $BaSO_4$		—	—	—	3.69
(7)	9.435	2.676 mg $BaSO_4$		—	—	—	3.89
Calc. for $C_{12}H_{16}N_4SO_2$-$2C_{10}H_8N_4O_5$				47.53	3.96	20.79	3.96
Calc. for $C_{12}H_{18}N_4SO_2$-$2C_{10}H_8N_4O_5$				47.48	4.20	20.74	3.95
Calc. for $C_{12}H_{16}N_4SO$-$2C_{10}H_8N_4O_5$ by Windaus & his co-workers.				48.48	4.04	21.21	4.04

Chloraurate :- Crystallises in light orange yellow long plates melting at 189°C (uncorr.) with decomposition; soluble in alcohol and acetone, sparingly in water, but insoluble in ether and benzene etc.. Dried at 100°C in vacuo and analysed;

Found No.	Subst mg	CO_2 mg	H_2O mg	Au mg	C %	H %	N %	S %	Cl %	Au %
(1)	4.373	2.469	0.754	1.829	15.39	1.92	—	—	—	41.42
(2)	4.573	2.530	0.802	1.895	15.08	1.94	—	—	—	41.44
(3)	6.229	0.312 cc N (16°C 758 mm)				—	5.83	—	—	—
(4)	5.673	0.273 cc N (16°C 758 mm)				—	5.69	—	—	—
(5)	9.393	2.274 mg $BaSO_4$		3.903 mg Au		—	—	3.32	—	41.56
(6)	9.620	2.672 mg $BaSO_4$		4.013 mg Au		—	—	3.81	—	41.72
(7)	4.717	5.575 mg AgCl		1.965 mg Au		—	—	—	29.23	41.65
(8)	4.550	5.440 mg AgCl		1.889 mg Au		—	—	—	29.56	41.52
Calc. for $C_{12}H_{16}N_4SO_2$-$2HAuCl_4$					15.00	1.83	5.83	3.33	29.58	41.04
Calc. for $C_{12}H_{18}N_4SO_2$-$2HAuCl_4$					14.97	2.08	5.82	3.33	29.52	40.96

The above figures agree closely with the formula $C_{12}H_{16}N_4SO_2$ (or $C_{12}H_{18}N_4SO_2$) which was forwarded in the previous paper[1] and agreed with the formulla $C_{12}H_{20}N_4SO_2$ given by VanVeen[2] except 4-atoms of hydrogen but differed from that $C_{12}H_{16}N_4SO$ ($+H_2O$) of Windaus and his co-workers[3] in the absence of crystal water.

(1) S. Ohdake: Proc. Imp. Acad. Japan, **7** (1931), 102~105; Ibid. **8** (1932), 179~182. Bull. Agr. Chem. Soc. Japan, **8** (1932), 11~46; Ibid. **8** (1932), 111~121, J. Agr. Chem, Soc. Japan, **7** (1931), 775~808; Ibid. **9** (1933) 185~197.

(2) VanVeen: Rec. Trav. Pay.-Bas, **50** (1931), 610; Ibid. **51** (1932) 279; Zeit. Physiol. Chem. **208** (1933), 185~197.

(3) A. Windaus, R. Tschesche, H. Ruhkopf, F. Laquer, & F. Schultz: Nachrich. Göttingen, (1931), 207~213; Z. Physiol. Chem. **204** (1932), 123~128; A. Windaus, R. Tschesche, & H. Ruhkopf; Nachrich. Göttingen, (1932), 342~346.

Activity of the hyrochloride:– The antineuritic acivity of the hydrochloride was tested on albino-rats as well as on pigeons and compared it with the hydrochloride isolated from rice-polishings.

(1) When young rats weighing about 40～50 g were fed on the artificial diet consisting of 60% purified starch, 20% purified casein, 15% peanuts oil and 5% McCollum's salt mixture, supplemented daily with 0.4 g of autoclaved yeast and 3-drops of cod-liver oil, they developed the symptoms of B_1-deficiency usually in 4—5 weeks. By supplementing, however, with 0.001 mg of the hydrochloride per os daily they were quickly cured and grew healthy. (Chart. 1, Rat. No. 474,)

(2) Young rats fed on the same artificial diet as above, but supplemented daily with 0.001～0.002 mg of the hydrochloride from begining of the experiment, grew normally with perfect health for 50 days. From these results, it was observed that 0.001 mg of the hydrochloride is equivalent to the international standard unit. (Chart. 1)

(3) Pigeons fed on the same artificial diet, developed the typical symptoms of polyneuritis after about 3-weeks, but by injecting 0.005～0.01 mg of the hydrochloride daily, the severe symptoms were improved in 2～3 hrs. and perfectly cured in 1～2 days. (Chart. 2)

(4) The curative day-dose for pigeon was found to be 0.0025 mg as shown in the follwing table.

No.	Pigeon No.	Body-weight g	Days to polyneuritis.	Body-weight, suffering from polyneuritis, g	Dose injected mg	Days of protection	Day-dose mg
(1)	455	300	27	184	0.012	6	0.0020
(2)	453	282	30	205	0 010	3	0.0033
(3)	451	307	22	220	0.008	2	0.0040
(4)	487	320	32	202	0.008	3	0.0027
(5)	488	325	18	212	0 008	3	0.0027
(6)	490(I)	295	24	224	0.008	4	0.0020
(7)	490(II)	295	28	227	0.008	4	0.0020
(8)	491	295	32	209	0.008	3	0.0027
(9)	493(I)	307	16	206	0.008	5	0.0016
(10)	493(II)	307	21	190	0.008	4	0.0020
(11)	506	325	21	210	0.008	3	0.0027

Average, 0.0025 mg

These results lead to the conclusion that the antineuritic substance isolated from yeast is a sulphur compound having the formula $C_{12}H_{16}N_4SO_2$ and it is identical with that isolated from rice-polishings.

The author wishes to express his sincer thanks to Prof. U. Suzuki for his kind advise throughout the experiment and to Sankyo Company, Ltd, for kind supply of the material. Thanks are due to Messrs. M. Kamada and T. Yamagishi for their assistance both in chemical and biological experiments.

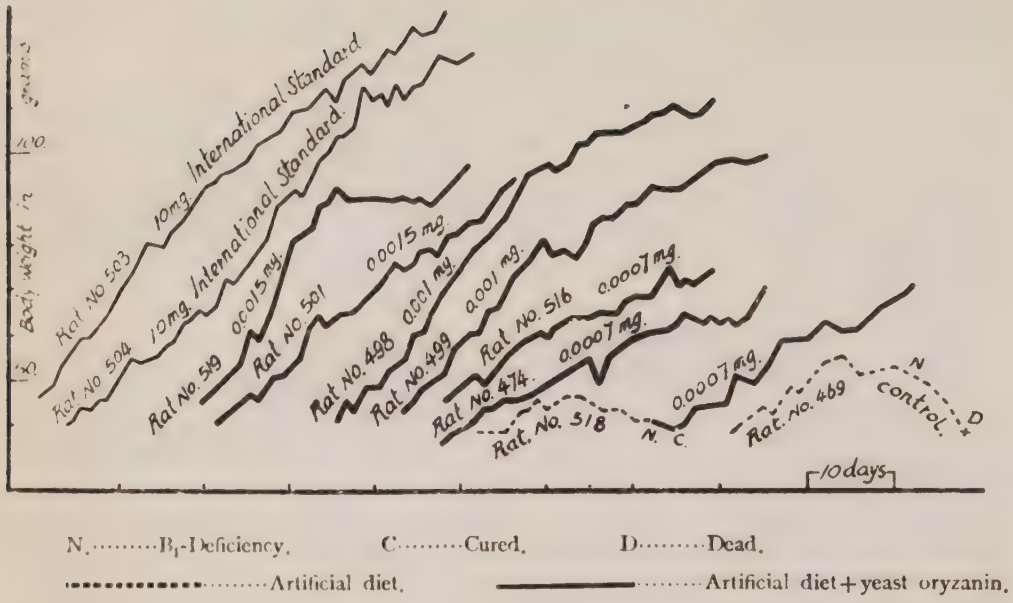

Chart. 1. Albinorats on artificial diet & yeast-oryzanin.

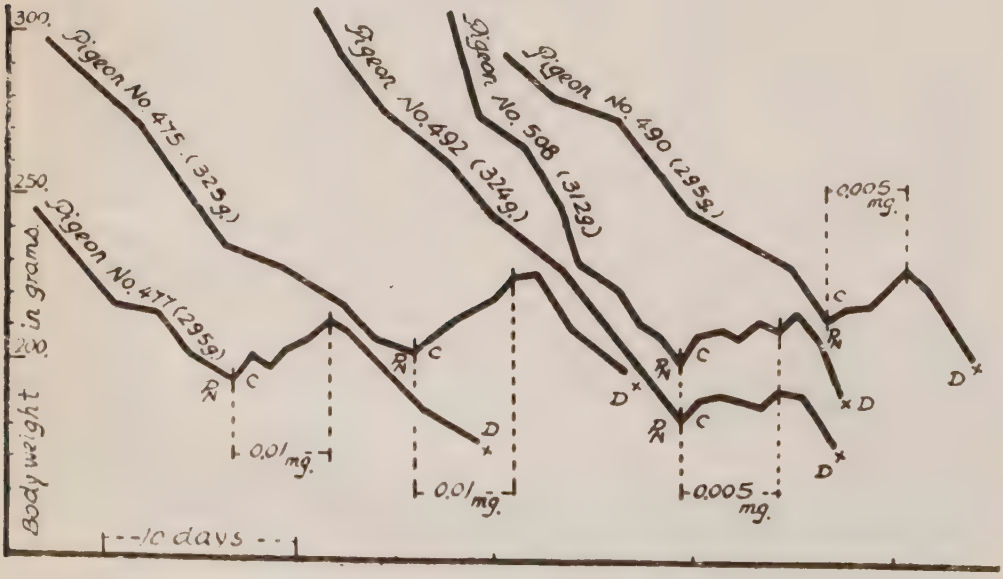

Chart. 2. Pigeons on artificial diet & yeast-oryzanin.

On the Organic Bases of the Flue-Cured Tobacco.

By

Takeo NITO.

(The Mito Experiment Station, Government Monopoly Bureau, Japan)

(Received April 5, 1934)

This investigation has been carried out to separate the organic bases from the flue-cured tobacco.

Total nitrogen, protein nitrogen and organic base nitrogen in the sample were determined as follows:—

	% in the dry matter
Total Nitrogen	2.453
Protein Nitrogen	0.854
Organic Base Nitrogen	0.525

About 1 kg of the tobacco leaves cut finely and treated with ether, was extracted three times with hot water. From 15 litres of the above gathered extracts, the protein and other impurities were removed by neutral and basic lead acetates, and excess of the lead in the filtrate by H_2S. The filtrate was evaporated under reduced pressure to almost equal to 1/50 volume of the original extract. After the removal of Nicotin, the precipitate of the organic bases formed by phosphotungstic acid, was fractionated into the following parts.

1. A_gNO_3-precipitate (purine Fraction).

From this fraction Adenine was isolated as hydrochloride. Yield: 0.25 g. Its picrate crystallised in long yellow needles and melted at 279°C. The chloroplatinate salt of the base formed yellow column crystals and on analysing it, the following result was obtained,

Subst. taken 0.0820 g Pt 0.0237 g Pt 28.90%
Calc. for Adenine-chloroplatinate $(C_5H_5N_5HCl)_2PtCl_4$ Pt 28.70%

2. A_gNO_3 and $Ba(OH)_2$-precipitate (Histidine and Arginine Fractions).

Histidine was clearly identified by Pauly's reaction but could not be isolated in crystalline form. And arginine was isolated as nitrate. Yield: 0.14 g. Its picrate crystallized in yellow needles and melted at 205°C. The coppernitrate salt of the base formed blue needle crystals and the analytical result is given below.

Subst. taken 0.0932 g. Cu 0.0109 g Cu 11.70%
Calc. for Arginine-copper-nitrate $[(C_6H_{14}N_4O_2)_2Cu(NO_3)_2]$ Cu 11.86%

3. Fitrate from $AgNO_3$ and $Ba(OH)_2$-precipitate (Lysine Fraction).

(a) Soluble part in cold absolute alcohol.

Any base was not detected.

(b) Insoluble part in cold absolute alcohol.

The picrate of the substance separated from this part crystallized in yellow columns and melted at 180°C. The chloroaurate of the base coincided with the Betaine-chloroaurate as follows:–

Subst. taken 0.0922 g Au 0.0401 g Au 43.49%
Calc. for Bataine-chloroaurate ($C_5H_{11}NO_2HCl$, $AuCl_3$) Au 43.13%

The organic bases (except Nicotine) isolated from about 1 kg flue-cured tobacco were as follow:–

Adenine (as hydrochloride)	0.25 g
Histidine	+
Arginine (as nitrate)	0.14 g
Betaine (as chloroaurate)	0.35 g

Biochemical Studies on "Sotetsu", the Japanese Sago Plant. I.

Changes of the General Composition of "Sotetsu"-Seed during its Ripening.

By

Kotaro NISHIDA

(*Kagoshima Agricultural College, Kagoshima, Japan.*)

(Received March 6, 1934)

The "Sotetsu" (*Cycas revoluta Thunb*) is a industrial crop for starch, and being resemble to the sago plant, is called "Japanische Sagopflanzen" by German. It is a special plant of Kagoshima- and Okinawa-prefectures in our country.

Experimental Results.

I. Weight Changes of Seed during its Ripening.

The auther used for experiment the materials, which are produced on

one "sotetsu"-plant growing in our college garden. The weight changes of the seed during its ripening are shown in the following table:

	30 Aug.	29 Sep.	28 Oct.	27 Nov.	27 Dec.	26 Jan.
Unhulled "sotetsu" (g)	13.25	17.30	17.75	18.00	17.65	17.36
Hulled "sotetsu" (Endosperm) (g)	5.65	9.40	10.35	10.20	10.20	10.18
Hull of "sotetsu" (Spermoderm) (g)	7.60	7.90	7.40	7.80	7.45	7.18

As is seen in the above table, the auther observed that the actual weight reaches its maximum during the ripening of "sotetsu"-seed, and then decreases in the later period.

II. Changes of Chemical Composition of seed during its Ripening.

The hulled "sotetsu" was dried and powdered; then its Chemical constituents were determined by the usual methods. The following figures are the percentage of the original weights of the hulled seeds:

	Aug. 30	Sep. 29	Oct. 28	Nov. 27	Oct. 27
Water	93.45	55.85	47.80	46.35	50.44
Dry matter	6.55	44.15	52.20	53.65	49.56
Total N	0.288	1.075	1.170	1.140	1.096
Protein N	0.145	0.942	1.075	1.103	1.084
Non-protein N	0.143	0.133	0.095	0.037	0.012
Starch	1.278	28.952	37.766	38.124	35.613
Dextrin		0.419	0.394	0.980	0.479
Reducing sugar	0.713	0.675	0.626	0.733	0.732
Non-reducing sugar	—	0.092	0.399	0.493	0.963
Crude protein	1.797	6.713	7.312	7.122	7.100
Crude fat	0.130	0.660	0.560	0.530	0.658
Crude fibre	—	1.290	0.926	0.875	0.802
Crude ash	0.476	0.880	1.081	1.038	0.981
N-free extract	4.148	34.607	42.322	43.885	40.020

III. Changes of Absolute Amount in one Seed during its Ripening.

From the above table, the absolute amount of chemical constituents in one "sotetsu"-seed was calculated as follows:

	Aug. 30	Sep. 29	Oct. 28	Nov. 27	Dec. 27
Water	5.280g	5.250	4.947g	4.728g	5.145g
Dry matter	0.370	4.150	5.403	5.472	5.055
Total N	0.016	0.101	0.121	0.116	0.112
Protein N	0.008	0.089	0.111	0.113	0.111
Non-protein N	0.008	0.012	0.010	0.003	0.001
Starch	0.072	2.721	3.909	3.889	3.633
Dextrin		0.039	0.041	0.100	0.049
Reducing sugar	0.040	0.063	0.065	0.075	0.075
Non-reducing sugar	—	0.009	0.041	0.050	0.098
Crude protein	0.102	0.631	0.757	0.726	0.724
Crude fat	0.007	0.062	0.058	0.054	0.067
Crude fibre	—	0.121	0.096	0.089	0.082
Crude ash	0.027	0.083	0.112	0.126	0.100
N-free extract	0.234	3.253	4.380	4.476	4.082

Summary.

According to the above analytical results it will be noted that:

(1) The dry matter, ash, protein, starch, dextrin and nitrogen-free extract content of the "sotetsu"-seed reaches its maximum distinctly at a definite point of the ripening period and decreases later.

(2) The sugar content of the "sotetsu"-seed increases gradually in the ripening period, on the other hand the content of fibre and non-protein nitrogen decreases gradually.

(3) In the ripening period of "sotetsu"-seed, the content of crude fat decreases gradually and increases later; but the crude protein comes up to its maximum and decreases later.

(4) The sample picked up on Aug. 30 is remarkably unriped seed, and the water content of the material is very large; then the reducing sugar, ash and non-protein nitrogen content in dried state is considerably high than that of other ripening date.

(5) From the above data, the weights of ripening endosperms and its compositions, the reasonable picking time of the "sotetsu"-seed for the raw material of starch manufacture seems to be the last decade of october to november at Kagoshima; but according to the climate even in the same district it may be more or less different.

Investigation on the Influence of Ultraviolet Rays on the Physiological Activities of Azotobacter.

I. On the lethal action of ultra-violet rays on Azotobacter chroococcum.

By

A. Itano and A. Matuura.

(Received March 10, 1934)

The lethal action of ultra-violet rays on Azotobacter chroococcum under various conditions was investigated by using Hanovia mercury lamp. First the quantity of ultra-violet rays, discharged from Hanovia lamp, was determined by both acetone methylene blue and molybdic acid methods, and found to be 3.0～2.5 discoloration in one hour and aH 2.1～1.6 in ten minutes respectively. The organism was exposed to the rays under various conditions, namely in Petri dish (hard glass of 29.126 transmission rate); under "Acme" ultravit-glass cover of 44.57 transmission rate and in Erlenmyer flask (hard glass of 57.888 transmission rate, measured against the quartz flask.), and obtained the following results:

Treatments	Time of exposure	Results
Petri dish	2 hrs.	Not killed
"Acme" ultravit glass	1 hr.	Majority killed
	2 hrs.	Totally killed
Direct exposure	30 seconds	" "
Quartz flask	5 minutes	" "
	5 seconds	Stimulated the growth
Erlenmyer flask, hard glass	2 hrs.	Not killed
	30～60 seconds	Stimulated the growth

Besides the viability of Azotobacter, the amount of nitrogen fixed and the change in the concentration of hydrogen ions under various treatments were examined. The lethal action of ultra-violet rays decreases in greater proportion than the rate of transmission of the apparatus. In all the cases, a short exposure stimulated the physiological activity of Azotobacter.

On the Effect of Linoleic Acid and Yeast upon the Growth of Rats on High Fat Diet.

By

Yoshikazu SAHASHI.

(Received January 20, 1934.)

In a previous communication[1] the present author reported the results of some feeding experiments on rats with synthetic diets containing large amounts of the oils obtained from sperm whale and fin-back whale.

Until recently, the chief physiological role assigned to dietary fats has been that of the fuel for the generation of energy, but certain unsaturated fatty acids are now recognized to be indispensable food factors like vitamins. The author, therefore, repeated some feeding experiments on rats using similar diets to those described in the previous paper but with the addition of linoleic acid and yeast.

Most of the rats in this experiment indicated almost normal growth, but no young were born during the entire feeding period except in the group supplied with butter. At the end of the experiment, the animals were killed and submitted to gross anatomical examination with the hope of determining the changes in sex glands. From this examination arose an unexpected discovery of the presence of vitamin E in Japanese soy bean oil, the details of which are given in a separate paper.[2]

Experimentel

(I) Samples af Fats or Oils used in the Animal Experiments.

Analysis of the samples used in the experiments:

Samples used	Sp. gr.	Refr. index	Acid value	Sap. value	Iod. value	Vitamin A Carotins
Butter (Kin en)	——	——	——	——	——	++
Beef fat***	——	——	0.6	196.7	48.2	–
Lard***	$d_4^{15} = 0.911$	$n_D^{26} = 1.471$	0.8	195.3	15.9	–
Cod liver oil (J. P. IV)	$d_4^{15} = 0.927$	$n_D^{22} = 1.475$	0.7	180.8	154.4	+++
Blubber oil* (Finback whale)	$d_4^{15} = 0.917$	$n_D^{22} = 1.466$	0.2	185.5	92.7	–
Intestine oil* (Finback whale)	$d_4^{15} = 0.916$	$n_D^{22} = 1.467$	0.9	180.6	97.6	—

These samples used in the experiments were kindly supplied by Toyo Hogei Kaisha* and Marumiya Company,** and the other ones*** were prepared from fresh raw materials in our laboratory.

Soy bean oil***	$d_4^{15} = 0.937$	$n_D^{30} = 1.481$	0.5	191.4	142.5	+
White sesame oil**	$d_4^{15} = 0.926$	$n_D^{23} = 1.471$	1.3	187.0	119.3	−
Peanut oil***	$d_4^{15} = 0.920$	$n_D^{30} = 1.475$	0.2	187.9	103.6	−
Cocoa-nut oil**	$d_4^{15} = 0.931$	$n_D^{23} = 1.452$	36.9	262.1	11.5	−
Palm oil*	—	—	—	—	—	+++
Olive oil (Betis spein)	$d_4^{15} = 0.919$	$n_D^{22} = 1.465$	0.9	191.1	92.9	−

(II) Experiment with Diets Containing a Sufficient Quantity of Fats or Oils, without the Supplementary Feeding of Linoleic Acid.

Data on the growth of rats on high fat diets without the supplementary feeding of linoleic acid will be presented first.

Albino rats weighing 40～50 g each were previously fed for several days on a complete diet until they reached 50～60 g. Then they were divided into several groups, each consisting of 4 rats, and were fed on various experimental synthetic diets (Charts 1 and 2). The diets consisted of:

Potato starch (Japanese Pharmacopea)	65 g	60 g
Fish protein freed from fat	18 g	15 g
McCollum's salt mixture	15 g	15 g
Oryzanin solution (Sankyo & Co.)	5 c.c.	—
Dried yeast (Oriental Co.) extracted with ether.	—	2 g
Fat or oil	15 g	20 g
Biosterin dissolved in olive oil, given per os daily	1 mg	1 mg

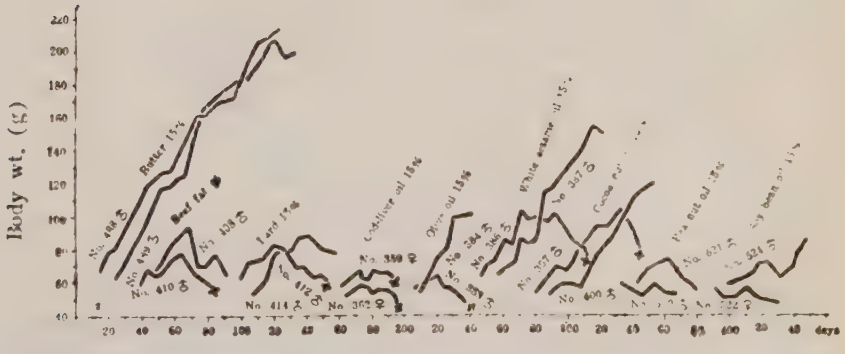

Chart 1. Growth curves of rats fed on the diets containing 15% fats or oils supplemented with oryzanin (without linoleic acid).

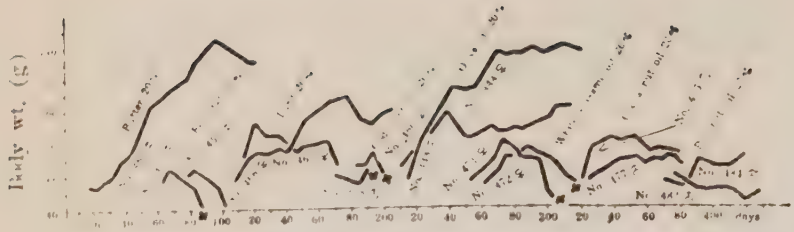

Chart 2. Growth curves of rats fed on the diets containing **20%** fats or oils supplemented with dry yeast (without linoleic acid).

From the above experiment it can be seen that rats failed to grow satisfactorily on any of the diets, except on the one containing butter. Slight seborrhea was observed in some of these rats (Photo. 3).

(III) Effect of Linoleic Acid and Yeast upon the Growth of Rats fed on High Fat Diets.

We now come to the main experiment which was conducted to see the effect of linoleic acid and yeast on the growth of rats fed on high fat diets, linoleic acid being now recognized as an indispensable food factor like a vitamin.

The diets used were similar to the preceding experiment: Potato starch 65 g, fish protein 15 g, McCollum's salt mixture 4 g, dry yeast extracted with ether 2 g, fat or oil 15～20 g, and in addition biosterin 1 mg and pure

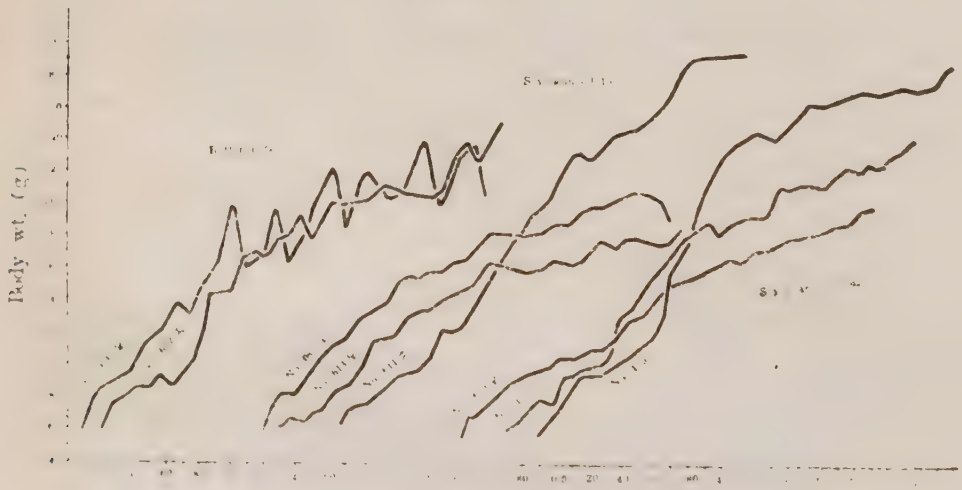

Chart 3. Growth curves of rats fed on the diets containing **15～20%** butter or soy bean oil supplemented with yeast and without linoleic acid. Linoleic acid was not added since butter as well as soy bean oil naturally contains this fatty acid.

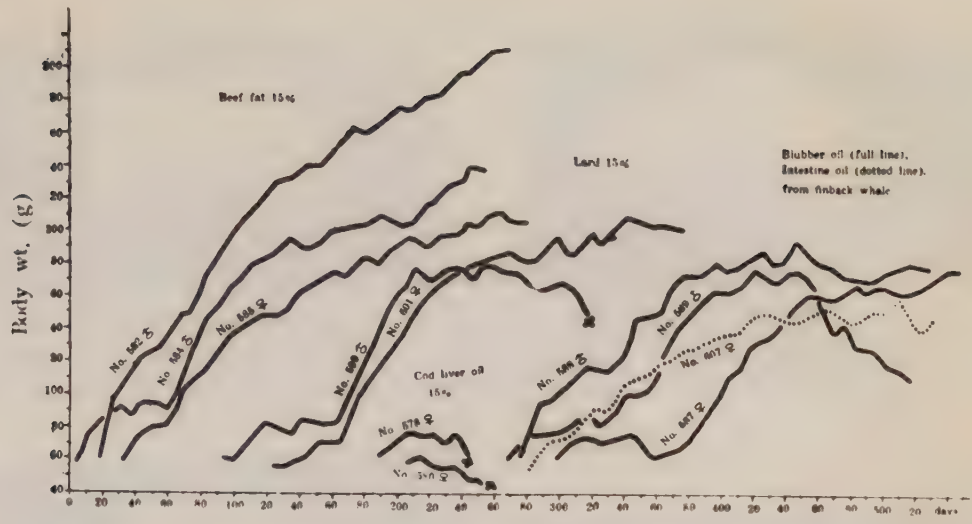

Chart 4 Growth curves of rats fed on the diets con aining 15% animal fats or oils supplemented with yeast and linoleic acid.

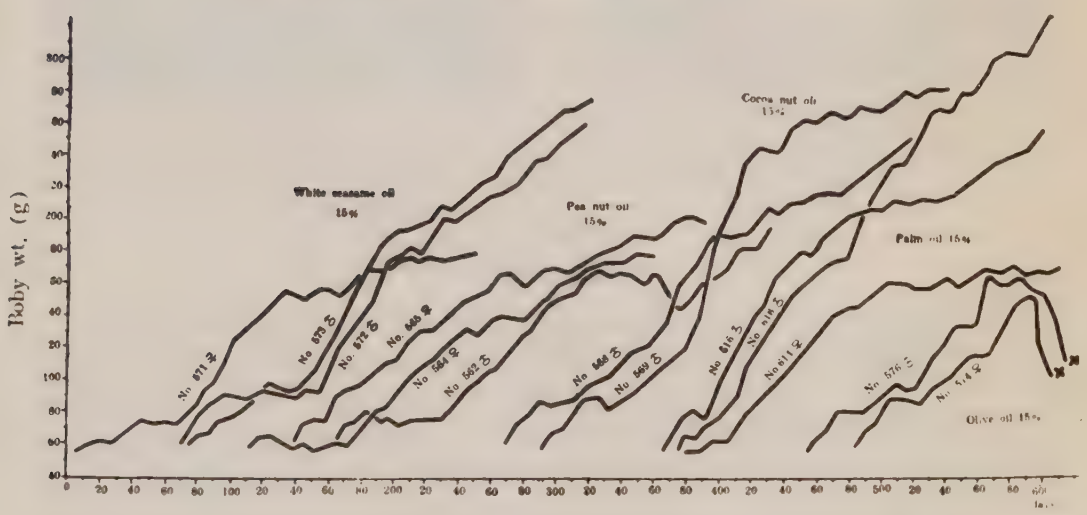

Chart 5. Growth curves of rats fed on the diets containing 15% vegetable fats or oils supplemented with yeast and linoleic acid.

linoleic acid 50～100 mg being supplied per os to every rat daily.

The rats given cod liver oil died after 50 days, but, on the contrary, those supplied with other oils and fats lived more than 250 days and secured nearly the normal growth (Charts 3～5 and Photo. 1, 2 and 4).

From the anatomical examination made on the rats at the termination of this experiment, the absence of vitamin E was recognized in all the above fats and oils except butter and soy bean oil. The evidence for the presence of vitamin E in soy bean oil is given in a separate paper.[6]

Photo. 1. Rat (No. 611 ♂) supplied with yeast in addition to 15% soy bean oil. Body weight 288 g at the 240 th day of experiment.

Photo. 2. Rat (No. 618 ♂) supplied with linoleic acid and yeast in addition to 15% palm oil. Body weight 302 g at the 210 th day.

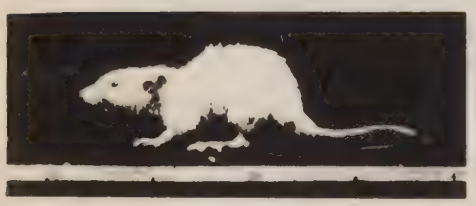

Photo. 3. Slight seborrhoea in rat (No.410 ♀) fed with beef fat without yeast or linoleic acid. Body weight 59 g at the 45 th day.

Photo. 4. Rat (No. 582 ♀) supplied with yeast and linoleic acid in addition to 15% beef fat. Body weight 310 g at the 240 th day.

Summary

Albino rats failed to secure satisfactory growth on diets containing a high percentage of any of the fats used except butter. The addition of yeast to the diet did not improve the growth of the animals. With the supplementary feeding of linoleic acid in addition to yeast the animals attained nearly the normal rate of growth, demonstrating anew the importance of linoleic acid as a dietary element.

The author expresses his sincere thanks to Prof. U. Suzuki for his kind advice and encouragement throughout the progress of this work.

Literature

(1) Y. Sahashi: Sc. Pap. I. P. C. R., **20**, 245~253, (1933); Bull. Agr. Chem. Soc., Vol. **9**, 69 (1933).

(2) U. Suzuki, W. Nakahara, and Y. Sahashi: Sc. Pap. I. P. C. R., **23**, No. 491, 270, (1934); Bull. Agr. Chem. Soc., Vol. **10**, 87 (1934).

The Occurrence of Vitamin E in Soy Bean Oil.

By

Umetaro Suzuki, Waro Nakahara and Yoshikazu Sahashi.

(Received January 20, 1934.)

Incidental to some animal experiments on the nutritional role of fats [1] an interesting observation was made that soy bean oil used as the only possible source of vitamin E prevented the testicular degeneration, which occurred consistently in animals receiving certain other oils. Although the general nutritive value of soy bean oil has already been investigated, the occurrence of vitamin E in this oil seems not to have been demonstrated.

In the present paper we wish to give a brief account of our preliminary experiment, which we consider sufficient to show that soy bean oil, unlike certain other vegetable oils, contains vitamin E.

Experiment

The composition of the diet used in our experiment was as follows:—

Potato starch (J. P)	65 g
Fish protein, freed of fat	15
Soy bean oil	15 or 20
McCollum's salt mixture	4
Dry yeast, extracted with ether	2

In addition biosterin, dissolved in olive oil, and pure linoleic acid were given separately to each animal in the daily dose of 1 mg and 50～100 mg respectively.

Soy bean oil was freshly expressed from soy beans in our laboratory, and it had the following properties:— Sp. gr., $d_4^{15}=0.937$; refr. index, $n_D^{40}=1.481$; acid value, 0.5; saponification value, 191.4; iodine value, 142.5. For comparison the following fats and oils were used in the same amount as and replacing soy bean oil:— Butter, blubber oil (fin-back whale), white sesame oil, peanut oil, cocoanut oil and palm oil.

Young rats were maintained on the above diet, with one of the oils as the source of fat. The rats grew nearly at the normal rate during the experimental period of 250 days, many attaining the body weight of 250～300 g. For the growth curves of these rats the reader is referred to a separate paper by one of us (Sahashi).

At the end of the experimental period of 250 days, there were 13 male rats distributed to different oil groups, and all these were killed and autopsy

performed.

No important pathological change was noted in any of the rats. Slight and localized inflammation in the lung, and a small number of tapeworm cysts in the liver were encountered in a few cases, but these findings are of no significance. We found, however, that testicles were in the state of very marked degeneration in many of the rats, as may be seen from their weights given in the following table:—

Table I.

Oils	Rat No.	Weight of testicle
Soy bean oil	478	1.10 g each
	479	1.20 g each
	611	1.20 g each
Peanut oil	562	0.40 g 0.50 g
Cocoanut oil	568	0.40 g each
	569	0.40 g 0.35 g
White sesame oil	572	0.55 g 0.60 g
	573	0.55 g 0.65 g
Palm oil	616	0.40 g each
	618	0.70 g each
Blubber oil	588	0.35 g each
	589	0.35 g each
Butter	602	1.10 g each

The degenerated testicles were not only small in size but were also very soft, not associated with signs of inflammation. In the accompanying photograph are shown three pairs of such degenerated testicles (lower row) in comparison with three other pairs (upper row) of perfectly normal testicles taken from the three soy bean oil rats. It is highly significant that testicles of the soy bean oil fed rats were absolutely normal in every way. Butter fed animal also showed normal testicles, but all the rest of the rats were suffering from marked testicular degeneration.

That so marked a degeneration of testicles should occur in spite of the generally excellent physical conditions of the rats is significant, and a consideration of the composition of the diet leaves little doubt that here we

Upper row of three pairs of normal testicles are from the rats fed with soy bean oil. In the lower row are three pairs of degenerated testicles from rats of cocoanut oil and peanut oil groups. The more or less uneven surface of the degenerated testicle is due to the shrinkage characteristic of such testicl s preserved in 10% formalin. Approximately natural size.

are dealing with the case of vitamin E deficiency. The large testicles, normal in every way, of the soy bean oil rats, then, constitute evidence that this oil contains vitamin E.

Additional experiments are now being conducted with the object of determining the effect of soy bean oil on the reproduction of rats. These experiments have not as yet progressed far enough to be published, but we have already obtained sufficient evidence to show that reproduction will take place on a synthetic diet with soy bean oil as the only possible source of vitamin E. We hope to give the details of these experiments in our next publication.

Conclusion

The incorporation of a liberal amount of soy bean oil to otherwise vitamin E deficient synthetic diet prevents the development of the testicular degeneration characteristic of vitamin E deficiency. This fact, taken together with certain evidence of fertility of rats on the same diet (unfinished experiments) leads us to conclude that soy bean oil contains vitamin E.

Literature.

(1) Y. Sahashi: Sc. Pap. I. P. C. R., **23**, No. 490, 264, (1934); Bull. Agr. Chem. Soc., Vol. **10**, 62 (1934).

Über die Verteilung der Manganverbindung im Boden Japans, und ihre Beziehung auf die Fruchtbarkeit.

Von

Chikafumi Ichikawa.

(Eingegangen am 19 Februar, 1934)

Mit 58 Proben des Bodens, die aus verschiedenen Provinzen in Japan gebracht worden waren, habe ich über die Enthaltung der Manganverbindung studiert.

Dabei ist sie in all diesen Proben betrachtet warden;

1. Der Enthaltungsgrad der von HCl gelösten Manganverbindung ist von Spur zu höchstens 0,612%, und desselbe der von KCl- lösung gelösten ist auch von Spur zu höchstens 30,67 mg in 100 g Probe.

2. Die von HCl oder KCl gelösten Manganverbindungen sehen im Boden aus jeder Provinz in Japan zu existieren aus.

Und der Enthaltungsgrad derselben hat ein Verhältnis zu die Fruchtbarkeit des Bodens.

3. Hierbei scheint es mir, je grosser der Vergleichsgrad zwischen die Quantität der von HCl gelösten Manganverbindung und dieselbe der von KCl gelösten ist, um so Fruchtbarer ist der Boden.

Researches on the Electric Boundary Layer Disturbance. Part IV

The Effect of the Alternating Electric Field on the Permeability Potential of Membrane

By

Masuzo Shikata and Koichiro Kitao

(The Chemical Laboratory of Forest Products, Kyoto Imperial University)

(Received May 25, 1934)

Prof. Michaelis has shown that the potential difference was always observed between each side of collodion membrane when the KCl solutions of different concentration was separated by this membrane.

He ascribed this phenomenon to the difference of mobilities of K ion and Cl′ ion through this membrane.

The authors have observed that if this membrane was once under the influence of an alternating electric field, for example during 10 to 30 seconds in the 220 A. C. field of 60 cycles, the potential difference was almost disappeared, and after 5 to 10 minutes it returned to the initial value.

Chemical Researches on the Pulp Woods in Karafuto. Part V

Cooking and Spinning Tests of Karafuto Fir

By

Masuzo Shikata and Takeo Kohsaka

(*The Chemical Laboratory of Forest Products, Kyoto Imperial University*)

(Received May 25, 1934)

The result of chemical analysis of the Akatodomatsu (*Abies sachliensis Fr. Schem.*) of Karafuto was given in table (I). No remarkable difference has been observed between three kinds of Akatodomatsu (i. e. three kinds; fir with long, medium and short bract-scales).

Although the total cellulose content of Karafuto fir is nearly the same as Karafuto spruce, Ezomatsu, (*Picea ajanensis Fisch.*), the alpha-cellulose content of the former is conspicuously less than that of the latter. We may conclude that the fir is much inferior to the spruce as the rayon pulp wood.

The result of cooking test is given in table (2). Ammonium bisulphite methods give better yields than magnesium bisulphite methods. The viscose silks have been prepared from these pulps and the Canadian Kipawa pulp. The tensile strength of these viscose silks have been tested.

Table I

The result of chemical analysis of the Akatodomatsu.

	fir with long bract-scales absolute dry%	fir with medium b. s. absolute dry%	fir with short bract-scales absolute dry%
Alcohol-benzene ext. substance	3.63	3.86	2.97
1%NaOH soluble matter	13.51	14.55	12.95

Hot water solu. matter	4.49	5.19	3.89
Cold water solu. matter	2.33	2.30	2.60
Total cellulose	55.35	55.47	55.23
Alpha-cellulose	34.82	34.84	33.89
Beta and gamma-cellulose	20.53	20.63	21.34
Lignin	30.21	30.42	29.07
Pentosan	10.99	11.60	13.68
Mannan	5.31	4.90	3.94
Galactan	0.25	0.25	0.23
Hemicellulose (10, 11, 12)	16.47	16.75	17.84
Methoxyl-radical	5.17	5.24	4.94
Nitrogen	0.08	0.08	0.07
Crude protein	0.51	0.48	0.46
Ash	0.41	0.42	0.40
Methoxyl-lignin ratio (%)	(17.12)	(17.20)	(17.00)

Table 2

Cooking and Spinning test

cooking solution	Mg-bisulphite	NH_4-bisulphite
Total SO_2	6.2%	6.2%
Base	MgO 1%	NH_3 1%
Maximum pressure	7 atm. p.	5.5 atm. p.
Mean pressure	4.5 atm. p.	4 atm. p.
Maximum temperature	155°C	145°C
Mean temperature	130°C	125~130°C
Time	10 hrs.	10 hrs.
Yield	35~36%	39~40%
Alpha-cellulose content in pulp	80%	75~80%
Tensile strength of the viscose silk	0.7 g/dn.	0.7 g/dn.

On the Production of Seborrhea in Rat by Feeding with whale Oil. Part I.*

By

Eiichi Somekawa.

(Received April 20, 1934.)

In the previous experiments, carried out by the author in cooperation with Dr. W. Nakahara, on the effect of various fats and oils upon the growth of rat tumor, It was observed that the rat when fed on a diet containing 10～15% whale oil (on sale containing about 40% unsaponifiable matter) develops seborrhea, characterized by the secretion of oily substance from the skin, the hair becoming completely imbibed with the oil so that the animal looks as if it were dipped in oil. The body weight gradually decreased and the animal finally succumbed within 2～3 weeks. The same disease is also produced by common sperm oil or arctic sperm oil.

As such a disease produced by whale oil is not yet recorded anywhere, the author has studied it more closely and tried to isolate the active substance which causes this curious disease. It is well known that sperm oil consists of mixed waxes, i. e. the mixed ester of cetyl alcohol, oleyl alcohol, etc. with fatty acids, such as palmitic, myristic, oleic etc. The solid wax which deposits on cooling the whale oil is cetyl palmitate, and the greater part of the liquid wax is presumed to consist of oleic acid ester of oleyl alcohol, i. e. oleyl oleate.

The author has observed that the characteristic sympton is no more produced when whale oil is saponified though the toxic property is increased. The author thus suspected that the liquid wax contained in whale oil might be the active substance. From such a point of view the author has prepared the oleic acid ester of oleyl alcohol synthetically and confirmed that exactly the same symptom is produced with this substance as with whale oil.

Y. Sahashi[1] in our laboratory has studied the same subject and observed that the solid wax (cetin, cetyl palmitate) has no toxicity because it is not assimilated at all, while oleyl alcohol has a very noxious action without producing seborrhea. It is in accordance with the author's view that the said disease is produced by the liquid wax (oleyl oleate) which on saponification loses the property of producing the disease though the saponified products act more noxiously.

* This paper was published in Sc. Pap. I. P. C. R., 21 (1933), 149—157.

Experimental

The blubber oil prepared from the bottlenose whale by Tōkai Fishery Company in Tateyama, Awa province, was used for the following experiment. It has the following properties:

Acid value	0.7
Iodine value	83.9
Saponification value	116.6
Unsaponifiable matter (%)	42.1

The composition of the diet was as follows:

Fish protein	15%
Starch	65%
Whale oil	**15%**
McCollum salt	5%
Oryzanin	5 cc
Riken-Vitamin A	1 drop per rat per day

When young male rats weighing 50~60 g were fed on the above diet, they could not grow at all, an oily substance diffused out over the skin, first around the anus and gradually extended over the whole body and at last the animals presented such an appearance as if they were dipped in oil (Table I, Fig. 1).

Table I.

Rat No.	Body weight (g)		Gain or loss in body weight (g)
	Initial	At 7 th day	
157	59	57 (Death)	− 2
158	56	54 (〃)	− 2
159	58	47 (〃)	−11
160	58	62	+ 4

In the next experiment, instead of oryzanin, 5 g of dry yeast were added per 100 g of diet. In this case the rat survived somewhat longer but suffered from the same disease as bofore (Table II. Fig. 2).

Table II.

Rat No.	Body weight (g)		Gain or loss in body weight (g)
	Initial	At 7 th day	
161	58	46	−12(Death at 9th day)
162	55	59	+ 4(〃 〃 〃 〃)
163	56	60	+ 4(〃 〃10th 〃)
164	56	66	+10(〃 〃11th 〃)

In the third experiment, 10 resp. 15% of dry yeast were added to the

diet as follows:

	(A)	(B)
Fish protein	15%	15%
Starch	55%	50%
Whale oil	15%	15%
Dry yeast	**10%**	**15%**
McCollum salt	5%	5%
Riken-Vitamin A	1 drop per rat per day	

After feeding 1～2 weeks on the above diets, the skin symptom appeared in both groups similarly. In group (A) no distinct symptom was observed for 5～8 drys, the growth was better than in group (B); but afterwards the disease developed rapidly and after 2 weeks it became quite the same as in group (B) (Table III, Fig. 3 and 4).

Table III.

	Rat No.	Body weight (g)		Gain or loss in body weight (g)
		Initial	At 14th day	
(A)	187	55	(Death at 8th day)	
	188	61	58	− 3
	189	60	65	+ 5
	190	60	68	+ 8
(B)	193	53	71 (At 7th day)	+18
	194	49	(Death at 6th day)	
	195	51	(〃 〃 5th 〃)	
	196	49	(〃 〃 7th 〃)	

We see from the above results that the noxious action of whale oil cannot be prevented even by adding 15% yeast to the diet.

On the whole, the symptom seemed to run parallel with the growth of rat; that is to say, the rat which grew relatively well, with large food intake presented more remarkable symptom.

Experiments with the unsaponifiable matter and fatty acids of whale oil.

(A) Saponification of whale oil: The oil was saponified by boiling with three times of its weight of 10% alcoholic potash for 2 hours, hereupon about one third of alcohol was distilled off, and after diluting with about five times of water the unsaponifiable matter was repeatedly extracted with ether until the ethereal soulution became colourless. The combined ether extract was then distilled and the residue thus obtained was saponified once more with a little alcoholic potash by warming for a short time. After diluting with water the unsaponifiable matter was extracted with ether as before, dehydrated with anhydrous sodium sulphate and the ether was distilled off.

The soap solution, from which the unsaponifiable matter had been removed, was acidified with dilute hydrochloric acid and the liberated free fatty acids were extracted with ether, washed with water, dehydrated and the ether was distilled off as before. W. Fahrion[2] has pointed out the presence of cetyl alcohol ($C_{16}H_{34}O$) in arctic sperm oil, and M. Tsujimoto[3] has shown that the unsaponifiable matters of sperm head oil and arctic sperm oil contain cetyl and oleyl alcohol ($C_{18}H_{36}O$).

(B) Separation of unsapnifiable matter: The separation of unsaponifiable matter into solid and liquid portions was effected by the method of Tsujimoto[1] as follows:—The unsaponifiable matter was dissolved in three times of its weight of acetone, cooled with freezing mixture for 1 hour and the separated crystalline substance was collected on a Buchner funnel and washed with a small amount of cold acetone. From the filtrate, after distilling off the acetone and cooling, a second crop of crystals was obtained. It was united with the first crystals and recrystallized twice from 80% alcohol or acetone; m p 49~50°.

The liquid portion was distilled at 13 mm, b p 170~208°; iodine value 85.5.

(C) Feeding experimets were now carried out with the unsaponifiable matter and fatty acids prepared as described above in order to determine which of the two is the active substance.

Composition of diets:

(A)

Fish protein	15%
Starch	75%
Fatty acids	**5%**
McCollum salt	5%
Oryzanin	5 c.c.
Riken-Vitamin A	1 drop per rat per day

(B)

Fish protein	15%
Starch	68%
Butter	10%
McCollum salt	5%
Solid unsap. matter	**2%**
Oryzanin	5 cc

(C)

Fish protein	15%
Starch	68%
Butter	10%
McCollum salt	5%
Liquid unsap. matter	**2%**
Oryzanin	5 cc

	(D)	(E)
Fish protein	15%	15%
Starch	70%	65%
Unsap. mattr + Fatty acids	**10%**	**15%**
McCollum salt	5%	5%
Oryzanin	5 cc	5 cc
Riken-Vitamin A	1 drop per rat per day	

The fatty acids, as well as the solid and liquid unsaponifiable matters added to the above diets (A), (B) and (C), were prepared from sperm head oil, while those prepared from arctic sperm oil, were used for (D) and (E).

Both of the oils contained about 40% unsaponifiable matter and 60%

fatty acids, and the unsaponifiable matter of sperm head oil contained roughly the same amount of solid and liquid portions, so that the contents of fatty acids and unsaponifiable matter in the diets (A), (B) and (C), were approximately equivalent to 10% of the original oil. This amount seems to be the minimum for producing the characteristic symptom on rat.

The sample used for group (D) and (E), was the mixture of 40% unsaponifiable matter and 60% fatty acids. The results of feeding experiments were as follows (Table IV):

Table IV.

	Rat No.	Body Weight (g)		Gain or loss in body weight (g)
		Initial	At 4 weeks	
(A)	73	47	73	+26
	74	52	(Death at 4th day)	
	75	51	(" " 14th ")	
	76	48	(" " 15th ")	
(B)	77	52	(Death at 15th day)	
	78	46	62	+16
	79	47	77	+30
	80	52	50	− 2
(C)	81	49	46	− 3
	82	51	(Death at 16th day)	
	83	48	61	+13
	84	47	66	+19
(D)	131	55	(Days survived) 10	
	132	56	14	
	133	52	12	
	134	52	4	
(E)	169	57	2	
	170	58	5	
	171	53	2	
	172	53	3	

It the above experiment, no characteristic symptom was developed in any groups, so it seems that this property is lost by saponification. However, the saponification products (fatty acids and wax alcohol) exerted more harmful effect than the original oil.

The experiment with the fatty acid ester of wax alcohol.

As described above, the saponification product, namely, fatty acids as well as the unsaponifiable matter, produced no characteristic symptom on

rats. However, the author suspected that the fatty acid ester of wax alcohol may exert the same action as whale oil and prepared synthetically the wax ester. Y. Toyama[5] has pointed out that the fatty acid of arctic sperm oil is largely the unsaturated acid of oleic acid series, so the author prepared the oleic acid ester of oleyl alcohol. Oleyl alcohol was prepared from the liquid fraction of the unsaponifiable matter by distilling two or three times fractionally under reduced pressure, and the fraction distilling at 8 mm, 193 ~200° was collected. The yield was more than 50% of the unsaponifiable matter; iodine value 90.46~92.21 (calc. 94.7). Its purity was further ascertained by elemental analysis (analyzed by Mr. Iki):

	Subst. (mg)	Co_2 (mg)	H_2O (mg)	C (%)	H (%)
found	4.470	13.140	5.518	80.17	13.81
calculated for $C_{18}H_{36}O$				80.94	13.10

Oleic acid used was Merck's preparation (extra pure). It was once more distilled under reduced pressure before using.

(A) Preparation of oleyl oleate: Oleic acid was converted into the acid chloride by treating with phosphorus trichloride. Twentyfive grams of oleyl alcohol were dissolved in a mixture of 50 cc of anhydrous benzol and 30 cc of anhydrous pyridine, and a slight excess of the chloride of oleic acid dissolved in 50 cc of anhydrous benzol, was added with cooling, allowed to stand at room temperature overnight, and after heating for 4 hours on the water-bath, benzol was distilled off under reduced pressure and the resulting reaction product was dissolved in petroleum ether (40~60°), washed with dilute sulfuric acid, made alkaline with 40~50% alcoholic potash and petroleum ether was renewed two or three times. The combined petroleum ether extract was washed one or two times with the above potassium hydroxide and then repeatedly with water, in order to remove the soap and alkali completely, and after dehydrating with anhydrous sodium sulfate, the solvent was distilled off in CO_2-atmosphere. The wax ester thus obtained, still contained some free oleyl alcohol and acid, so it was repeatedly washed with absolute alcohol, and a little alcohol adhered to the ester was expelled off by warming in vacuum. An almost colourless oily liquid was thus obtained; yield 40 g (ca. 80% of the theory); saponification value 105.65 (calc. 105.25). This sample was analyzed by Mr. Iki with the following result:

	Subst. (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
found	3.391	10.01	3.98	80.51	13.13
calculated for $C_{18}H_{35}O\cdot CO\cdot C_{17}H_{33}$				81.20	12.78

(B) Feeding experiments were carried out with the synthetic oleyl oleate prepared as described above:

Composition of diet:

Fish protein	15%
Starch	65%
Oleyl oleate	**15%**
McCollum salt	5%
Oryzanin	5 cc
Riken-Vitamin A	1 drop per rat per day

By feeding on the above diet, an oily substance diffused out over the skin around the anus of rat after 1～2 days, and finally the animals developed exactly the same symptom as in the case of whale oil. The similar experiment was repeated with the crude ester prepared by different methods with the same result. Oleyl palmitate and cetyl oleate, prepared by the above-mentioned method, did not show any distinct effect on rat, a slight symptom appearing at the beginning disappeared quickly (Table V, Fig. 5).

Table V.

Rat No.	Body weight (g)		Gain or loss in body weight (g)
	Initial	At 10th day	
203	59	55	− 4
204	58	51	− 7
205	62	59 (7th day)	− 3

From the above results it was proved that the synthetic oleyl oleate has the same action as whale oil.

Summary

1. When rats were fed on sperm head oil as well as on arctic sperm oil (oils of skin, muscle and bone), adding 10～15% in the diet, an oily substance diffused out from the skin and the animals show such an appearance as if they were dipped in oil. Besides, the above oils are very noxious, retarding the growth and finally causing the death of animals in a few weeks.

2. The above symptom produced by the whale oil cannot be prevented by adding 15% yeast in the diet, though the toxicity seems to be somewhat reduced, but rather the addition of yeast favor the development of more conspicuous symptom.

3. Saponification products of whale oil, namely, fatty acids and unsaponifiable matter, produced no such symptom as the original oils, but the toxicity was stronger than the latter. Moreover, it is not decided whether or not the substance which causes seborrhea is identical with that which prevents the growth of the animal.

4. The severe symptom appeared on these animals which grew relatively well with good food intake.

5. The oleic acid ester of oleyl alcohol synthetically prepared by the author exerted the same effect as whale oil, but oleyl palmitate or cetyl oleate gave no such phenomena.

In conclusion, the author wishes to express his sincere thanks to Prof. U. Suzuki for his kind guidance. Thanks are also due to the Tōkai Fishery Company and to Mr. K. Tashima for their kindness in supplying the materials.

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Fig. 1—Blubber oil of bottlenose whale 15%, 7th day. No. 160.

Fig. 2—Yeast 5 g per 100 g of diet (whale oil 15%), 8th day. 161.

Fig. 3—Yeast 10% (whale oil 15%), 13th day. No. 190.

Fig. 4—Yeast 15% (whale oil 15%), 7th day. No. 193.

Fig. 5—Synthetic oleyl oleate 15%, 10th day. No. 203.

Fig. 6—Normal rat.